Appendix D

PREPARATION OF NON-ENDOGENOUS VERSIONS OF GPCRS

TDAG8

Preparation of the non-endogenous, constitutively activated human TDAG8 receptor was accomplished by creating an Isoleucine to Lysine mutation at amino acid 225 (I225K). Mutagenesis was performed using Transformer Site-Directed™ Mutagenesis Kit (Clontech) according to manufacturer's instructions. Two mutagenesis primers were utilized, a lysine mutagenesis oligonucleotide (which included a coding sequence for lysine, AAA, as underlined below) and a selection marker oligonucleotide, which had the following sequences:

5'- GGAAAAGAAGAGAATCAAAAAACTACTTGTCAGCATC -3', and

5'- CTCCTTCGGTCCTCCTATCGTTGTCAGAAGT -3',

respectively.

GPR35

Preparation of the non-endogenous, constitutively activated human GPR35 receptor was accomplished by creating an Alanine to Lysine mutation at amino acid 216 (A216K). Mutagenesis was performed using Transformer Site-Directed™ Mutagenesis Kit (Clontech) according to manufacturer's instructions. The two mutagenesis primers were utilized, a lysine mutagenesis oligonucleotide (which included a coding sequence for lysine, AAA, as underlined below) and a selection marker oligonucleotide, which had the following sequences:

5'- GCCACCCGCAAGGCTAAACGCATGGTCTGG -3', and

5'- CTCCTTCGGTCCTCCTATCGTTGTCAGAAGT -3',

respectively.